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New Phenomenon

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Soluble (pro)renin receptor as a novel laboratory biomarker of atherosclerosis

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Atherosclerosis (AS), a chronic inflammatory disease involving the large and middle arteries, is characterized by inflammation, abnormal deposition of lipids, and other pathological events [1]. Endothelial cell injury, the migration and proliferation of vascular smooth muscle cells, and the inflammatory polarization of macrophages play crucial roles in the formation and progression of atherosclerotic plaques [1]. The renin–angiotensin system (RAS), an essential regulator of the inflammatory response, is closely correlated with atherosclerotic plaque formation [2]. Targeting the components of the RAS could be a promising therapeutic strategy for AS.

(Pro)renin receptor (PRR), a single transmembrane protein, works as a key regulator of the local RAS with nearly equal affinity to bind to renin and (pro)renin and plays essential roles in cardiovascular homeostasis by targeting multiple RAS-dependent and RAS-independent intracellular signals in cardiovascular cells [3]. sPRR, a soluble form of PRR, is generated by proteases (Furin, a disintegrin and metalloproteinase 19, site-1-protease, or an unknown convertase)-mediated cleavage of the full-length PRR and is released into extracellular spaces, including the plasma and urine, where it participates in various physio-pathological processes [4]. An increasing number of studies have demonstrated an increase in circulating sPRR levels in patients and animals with various cardiovascular diseases, including hypertension and heart failure, which may be promising indicators of these cardiovascular diseases [4]. Amari *et al.* [5] reported that serum sPRR levels were significantly greater in hemodialysis patients with an ankle–brachial index (ABI) < 0.9 (an indicator of severe AS or obstruction of lower limb arteries) than in patients with an ABI ≥ 0.9. They reported a negative correlation between serum sPRR levels and ABI independent of other atherogenic risk factors, including age and hemoglobin A1c, suggesting an association between serum sPRR levels and severe AS of the lower limbs [5]. Thus, increased serum sPRR levels may be a marker for AS progression. However, the clinical significance of the sPRR in patients with AS remains unclear.

To further determine the association between plasma sPRR levels and the severity of AS, we prospectively enrolled 236 participants in the present study, including 63 subjects with non-AS (n = 63, 58.8 ± 11.7 years) and 173 with AS (n = 173, 67.1 ± 11.0 years, P < 0.001 vs the non-AS group). The diagnosis of subclinical atherosclerosis was established after the carotid intima–media thickness and plaque area were evaluated via bilateral carotid ultrasonography. Patients with heart diseases (rheumatic heart disease, valvular heart disease, and cardiomyopathy), hepatic failure, chronic kidney disease, hyperthyroidism, chronic inflammatory disorders, malignancy, or pulmonary embolism were excluded. The baseline clinical characteristics are shown in Supplementary Table S1. Although plasma brain natriuretic peptide, urea nitrogen, and triglyceride levels were slightly higher in the AS group than in the non-AS group, there were no significant differences in sex, body mass index, comorbidities, blood pressure, parameters reflecting cardiac function, and plasma creatine, uric acid, low-density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), and total cholesterol between the two groups. The ApoE$^{-/-}$ mice were fed with a high-fat diet (HFD) for 16 weeks to establish an atherosclerotic mouse model successfully, which was determined by Oil-red-O staining of the full-length aorta and the aortic root as well as H&E staining of the aortic root (Supplementary Figure S1).

The studies involving human participants were reviewed and approved by the Affiliated Hospital of Jiangxi University of Chinese Medicine (JZFYLL20230208002). The patients/participants provided written informed consent to participate in this study. The

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studies involving animals were reviewed and approved by the Animal Care and Use Committee, which approved the animal protocols at Jiangxi University of Chinese Medicine (No. JZLLSC20230254).

The levels of plasma sPRR in AS patients were significantly higher than those in the non-AS groups (15.9 ± 6.9 vs 11.5 ± 4.0 ng/mL, $P < 0.001$, Figure 1A). After HFD feeding for 16 weeks, the levels of plasma sPRR in ApoE$^{-/-}$ mice were also significantly greater than those in ApoE$^{-/-}$ mice fed with a normal diet (24.1 ± 8.6 vs 10.3 ± 3.1 ng/mL, $P < 0.001$; Figure 2A). Thus, plasma sPRR levels are significantly elevated under atherosclerotic conditions. To our knowledge, several reports have indicated a potential relationship between PRR or sPRR and AS. A series of studies demonstrated that PRR regulates LDL metabolism by controlling the abundance of LDL receptor (LDLR) and sortilin-1 [6,7]. In this context, hepatic-specific PRR inhibition via N-acetylgalactosamine-modified PRR antisense oligonucleotide (GalNAc-PRR ASO) decreased plasma LDL levels by inhibiting lipid synthesis and uptake and promoting fatty acid oxidation, thus protecting against HFD-induced obesity and hepatosteatosis [7]. Hepatic-specific PRR inhibition accelerated AS in HFD-fed LDLR$^{-/-}$ mice but did not affect AS in HFD-fed ApoE$^{-/-}$ mice, which may be explained by the increase in proinflammatory

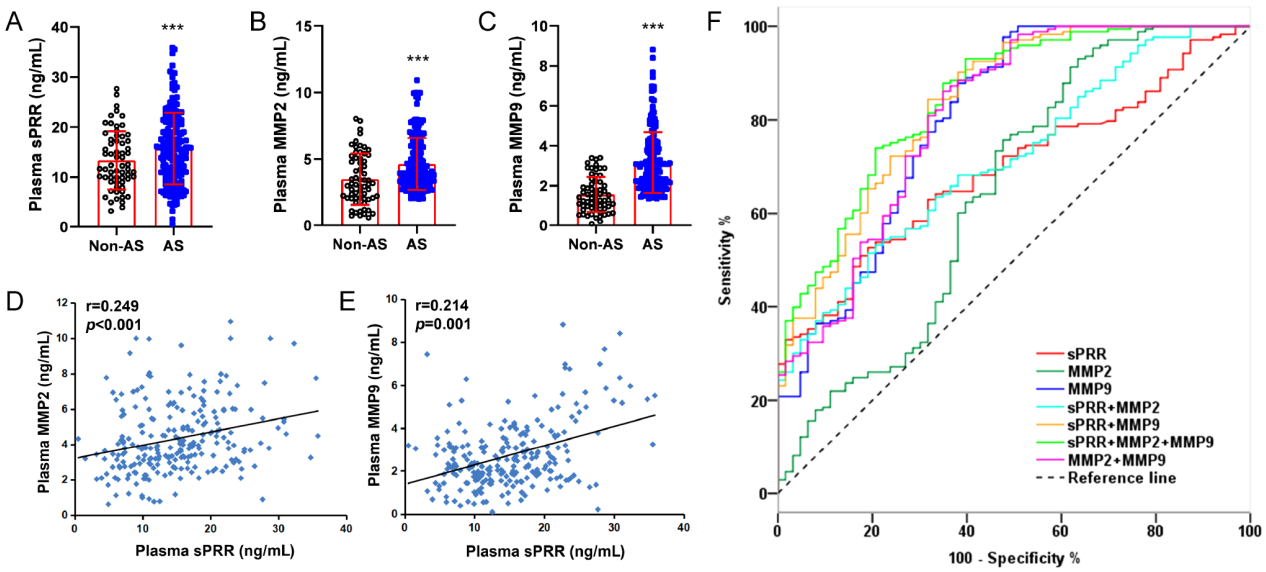


Figure 1. Plasma soluble (pro)renin receptor (sPRR) levels in patients with atherosclerosis (AS) (A–C) Plasma sPRR and matrix metalloproteinase 2/9 (MMP2/9) levels were assessed via ELISA. $n = 63$ for the non-AS group and $n = 173$ for the AS group. ***$P < 0.001$ vs non-AS patients. (D,E) Correlations between plasma sPRR and MMP2/9. (F) Receiver operating characteristic (ROC) curves of sPRR, MMP2, and MMP9 levels as well as their combinations for diagnostic value for atherosclerosis.

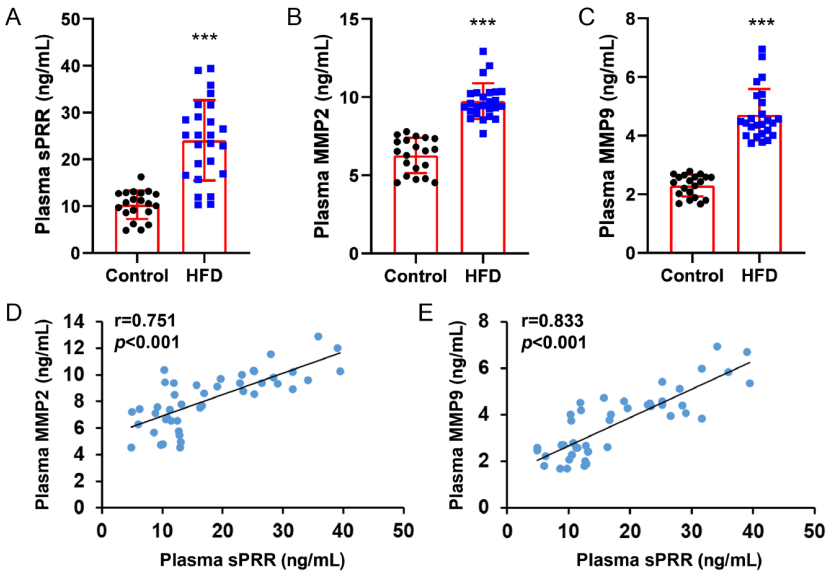


Figure 2. Plasma soluble (pro)renin receptor (sPRR) and matrix metalloproteinase 2/9 (MMP2/9) levels and their correlations in high-fat diet (HFD)-fed ApoE$^{-/-}$ mice $n = 20$ for the control group and $n = 25$ for the HFD group. ***$P < 0.001$ vs Control.

responses in macrophages caused by PRR inhibition in these mice [8]. This finding was supported by the observations that macrophage-specific deletion of PRR also exacerbates AS in HFD-fed ApoE$^{-/-}$ mice [8]. Although systemic *PRR* knockdown by subcutaneous injection of PRR-siRNA cannot significantly improve AS in HFD-fed LDLR$^{-/-}$ mice, it ameliorates AS in HFD-fed ApoE$^{-/-}$ mice, accompanied by reduced plasma LDL and triglyceride levels (https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CMFD202002&filename=1019909467.nh). Thus, the effect of PRR on AS may exhibit histocyte and species differences, which may be independent of its regulation of lipid metabolism. This finding may also imply that the impact of sPRR on AS is not related to lipid metabolism, although a significant increase in plasma sPRR levels was consistently observed in HFD-fed C57BL/6J mice [9,10] and HFD-induced atherosclerotic ApoE$^{-/-}$ mice in the present study. Although sPRR is also adversely linked to lipid metabolism in mice, as demonstrated by Wu *et al.* [9], who used adipocyte-PRR-deficient mice, the impact of sPRR on AS progression as well as the source of plasma sPRR under atherosclerotic conditions remains unknown. Studies employing the site-1 protease inhibitor PF429242 or mutagenesis of the cleavage site of PRR to inhibit endogenous sPRR generation and exogenous recombinant sPRR protein infusion or tissue/cell-specific sPRR overexpression to construct a high-sPRR model are recommended in the setting of AS.

Matrix metalloproteinase (MMPs), also known as matrixins, are biomarkers of atherosclerotic plaque instability and can be secreted by various cells present in the vascular wall to degrade extracellular matrix (ECM) proteins in the extracellular environment and promote the initiation, progression, and rupture of atherosclerotic plaques [11]. Here, we found that the mean plasma MMP2 (Figure 1B) and MMP9 (Figure 1C) levels in the AS patients were significantly higher than those in the non-AS patients. Similar results were also obtained in HFD-induced atherosclerotic ApoE$^{-/-}$ mice, in which the mean plasma MMP2 (Figure 2B) and MMP9 (Figure 2C) levels were also significantly elevated compared with those in ApoE$^{-/-}$ mice fed with a normal diet.

To estimate the diagnostic values of sPRR, MMP2/9, and their combinations for AS, the area under the receiver operating characteristic (ROC) curve (AUC) was analyzed for data from all non-AS and AS patients (Figure 1F and Supplementary Table S2), followed by a pairwise comparison of ROC curves via the DeLong test (Supplementary Table S3). A significant difference was found between the AUC values of sPRR + MMP9 or sPRR+MMP2+MMP9 and those of the other variables (Supplementary Table S3). When a single diagnosis was used, MMP9 had the highest diagnostic value, followed by sPRR and MMP2, suggesting a potential diagnostic value of plasma sPRR levels for AS with moderate sensitivity and specificity. When multiple joint diagnoses were performed, sPRR combined with MMP9 alone or MMP2 plus MMP9 had the highest and comparable diagnostic value. Thus, a combined assessment of sPRR and MMP2/MMP9 may be a good choice to increase the accuracy of the diagnosis of AS.

We divided all the AS patients into high-sPRR-level and low-sPRR-level groups on the basis of the optimal cutoff point (15.07 ng/mL) of the sPRR obtained from the ROC curve (Supplementary Table S4). Although most parameters did not differ significantly between the two groups, the right carotid plaque area and left ventricular end-diastolic diameter (LVEDd) were significantly greater in the high-sPRR-level group than in the low-sPRR-level group. These results revealed a possible association between increased plasma sPRR levels and plaque size and cardiac dysfunction. In this context, increased plasma sPRR levels may be a potential index for AS progression. We further analyzed the correlation between the plasma sPRR and the study variables in all the subjects using the spearman correlation analysis (Table 1). Plasma sPRR levels were positively correlated with MMP2 levels (Figure 1D), MMP9 levels (Figure 1E), HDL-c levels, the left atrial diameter (LAD), the left carotid plaque area, and the right carotid plaque area. We also analyzed the correlation between plasma sPRR and plasma MMPs in HFD-induced atherosclerotic mice. The

Table 1. Spearman correlation between sPRR and study variables in all subjects

Parameters	*r* value	*P* value
Age, years	−0.013	0.844
Gender	0.030	0.646
Body mass index, kg/m^2	0.305	0.474
Arrhythmia	0.384	0.553
Ischemic cerebrovascular disease	0.043	0.483
Hypertension	0.029	0.763
Systolic blood pressure, mmHg	0.034	0.599
Diastolic blood pressure, mmHg	0.050	0.447
Mean arterial pressure, mmHg	0.050	0.442
Heart rate, bpm	−0.077	0.242
Plasma MMP2, ng/mL	0.249	< 0.001***
Plasma MMP9, ng/mL	0.214	0.001**
Plasma BNP, pg/mL	0.033	0.618
Plasma creatine, μM	0.048	0.461
Plasma urea nitrogen, μM	0.127	0.051
Plasma uric acid, μM	0.017	0.796
Plasma LDL-c, mM	0.031	0.641
Plasma HDL-c, mM	0.146	0.025*
Plasma triglyceride, mM	−0.085	0.195
Plasma total cholesterol, mM	0.072	0.270
LVEF, %	−0.072	0.273
LAD, mm	0.130	0.046*
LVEDd, mm	−0.053	0.415
LVEDs, mm	0.036	0.577
IVST, mm	0.036	0.582
LVPW, mm	−0.043	0.515
RVIDd, mm	−0.004	0.955
Left carotid maximum IMT, mm	0.035	0.594
Right carotid maximum IMT, mm	0.012	0.860
Left carotid plaque area, mm^2	0.177	0.006**
Right carotid plaque area, mm^2	0.148	0.023*

BNP, B-type natriuretic peptide; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; LAD, left atrial diameter; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; IVST, interventricular septum thickness; LVPW, left ventricular posterior wall thickness; RVIDd, right ventricular internal dimension diameter; AS, atherosclerosis; IMT, intima-media thickness; MMP2/9, matrix metallopeptidase 2/9. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

plasma sPRR levels were also positively correlated with the MMP2 levels (Figure 2D) and MMP9 levels (Figure 2E) in these mice. These results suggest that a certain association may exist between plasma sPRR levels and plaque vulnerability and that increased plasma sPRR levels may lead to a tendency toward vulnerable plaques. In angiotensin II-induced abdominal aortic aneurysm (AAA) in ApoE$^{-/-}$ mice, PRR overexpression markedly increased, whereas PRR inhibition significantly blunted MMP2/9 expression/activity, suggesting that PRR-stimulated MMP2/9 may be responsible for the degradation of the ECM and vascular rupture in an AAA model [12]. However, our current data are insufficient to support a positive relationship between plasma sPRR levels and the stability of atherosclerotic plaques. Additional data from imaging techniques, including intravascular ultrasound or optical coherence tomography, which can define plaque structure, are needed. On this basis, the associations between plasma sPRR levels and the lumen, external elastic membrane, and plaque cross-sectional area in AS patients can help strengthen the correlation of sPRR with plaque vulnerability.

Multiple linear regression analyses of all participants were further performed to determine the relationships between plasma sPRR levels and the clinical characteristics associated with AS (Supplementary Table S5). We found a significant association between plasma sPRR levels and plasma MMP2, HDL-c, LAD, and the left carotid plaque area, revealing that the MMP2/9, HDL-c, LAD, and carotid plaque area may be independent predictive factors of the plasma sPRR levels in the context of AS. Using univariate and multivariate logistic regression analyses, we further investigated the clinical characteristics associated with AS, which may be the underlying risk factors for AS development (Supplementary Table S6). In both the univariate and multivariate analysis, plasma sPRR, MMP2, and MMP9 levels were consistently associated with the occurrence of AS. These results indicated that increased sPRR levels may be an underlying indicator of AS progression.

In summary, our study, for the first time, revealed a positive correlation between plasma sPRR levels and the severity of AS in humans and mice. The sPRR may be a useful laboratory biomarker for diagnosing AS.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biphysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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